OBSERVATIONS ON THE PARASITISM OF ANODONTA PLANA LEA BY A DISTOMID TREMATODE, AT CHAUTAUQUA, NEW YORK.

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THE materials on which these observations are based were collected at Chautauqua Assembly on the south shore of Lake Chautauqua, New York, during the months of July and August of 1895, 1896, and 1897. The animals are very abundant in the semi-muddy bottoms near the shore, and can easily be seen and watched *in situ* and reached and collected with the hand from a boat. They are found in company with several species of Unio, and it is not always possible to tell from the boat whether one has found this genus or Unio. The studies were made partly in the Biological Laboratory of the Chautauqua College of Liberal Arts and partly at Hamline University, Saint Paul, Minn. Most of these shells of Anodonta exhibit on the inner surface a more or less extensive vermilion-yellow-ochre coloration, in the form, apparently, of a foreign material laid down at the expense of the nacre. This led me to study the case carefully, and brought to light the fact that the red-colored cases of Anodonta are infested by a distomid parasite which lives in the space between the mantle and the shell, and is apparently the agent chiefly concerned, directly or indirectly, in the production of the red coloration. A somewhat careful examination of the materials at hand has brought certain facts to light which seem of interest as new or little known.

The fact that shells of Anodonta are reddened has been known since 1839, when Lea[1] was misled into describing shells thus diseased from Ohio as a new species, *Anodonta salmonea*, in language which so closely agrees with the case of the Chautauqua shells as to leave no doubt of the identity of the two. But so far as I have been able to ascertain, the colora-

[1] Lea, Trans. Am. Phil. Soc. N.S., vol. vi, p. 45. Pl. XIV, Fig. 41. 1839.

tion has never before been traced to its cause, nor have the flukes in this situation been noticed and described, and hence a careful account of the matter is desirable. I am indebted to Mr. Chas. I. Simpson of the United States National Museum for the identification of the Anodonta, and for the information about Lea's work, and to Dr. W. S. Nickerson of the University of Minnesota for suggestions in connection with the identification of the fluke.

I. THE EXTENT OF THE COLORATION.

The amount of the deposited salmon-tinted material is very variable indeed. I will describe an extreme maximum case at the outset. The shell in this instance has an extreme length of 62 mm. The entire inner surface, excepting the pallial line and the muscular impressions, is salmon colored; this includes the area between the pallial line and the borders of the shell, and all of the hinge area. The only points at which the bluish native color of the shell can be seen are the muscular impressions and the pallial line. The shell is thicker than normal, and the red is shown to be an abnormally thick deposit by the fact that the muscular impressions and the pallial line, instead of being flush with the inner surface, are beneath the general level, the pallial line forming a very conspicuous furrow, as in many of the Unios with their much thicker shell. The red layer, moreover, is not smooth, but minutely warty-roughened, and in many instances increased by folds. Its luster is dull or in places distinctly pearly, especially toward the borders of the shell, *i. e.*, in the newest portions of the deposit.

An extreme minimum stands in marked contrast with this maximum case. Such a shell (described from one actual case) is 75 mm. long; its general inner surface is perfectly normally bluish tinted, with mother-of-pearl luster, thin and perfectly smooth. The only indication of the red coloration is a small, thin, very slightly granular area of red 5 mm. across, which lies directly beneath the umbo and is of the same extent and position in both valves. Between these extremes of little and much coloration I have found every intermediate amount.

Moreover, the color is not indiscriminately scattered as if merely accidentally, but it is very definite in its distribution in two respects; namely, in the single valve and in both valves.

First in one valve it appears to be a law that the location of the coloration varies with the amount of the red material. Thus (*a*) in case of a very small deposit this is found directly beneath the umbo of each valve and in no place else; (*b*) in case of a larger deposit the color is found in a patch which extends from the umbo downwards and forwards towards the ventral border of the shell; (*c*) in case of still more red there is a second narrower patch running down posteriorly from the umbonal area toward the ventral border of the shell; (*d*) in cases of still more coloration these areas are filled in between so that in some cases the red crosses the shell above, but not ventrally, and in others it extends to the pallial line, or even beyond this to the very margin of the shell (see Fig. 1).

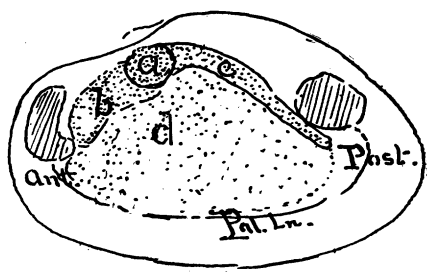

FIG. 1. — Inner surface of valve of Anodonta. The dotted lines indicate areas affected by the color-deposit.

It will be noted that these differences are not progressive stages observed on a single individual, but they are different cases that are observed. They may, however, indicate a progress in the growth of the coloration; if so, then we should have to say that the process begins at the umbo, then spreads in a narrow line downwards and outwards, first anteriorly, then posteriorly, and that later it spreads over the whole shell from these points. While I do not know of any actual evidence to show that such is the case, still the facts indicate some such order.

Not only is the coloration of the single valve thus definite, but it is an even more remarkable fact that the effect is uniform on the two valves; that is, the amount and position of the coloration is always bilaterally symmetrical. If one valve has a small spot at the umbo, then so has the other; there is

always an anterior strip in both valves if there is in one, and so on. In some cases the amount of the red color is so great that the shell lining is thrown into folds; in such a case we find folds on both sides, but they are not symmetrical, and in this minor point the symmetry is slightly lost.

It will be interesting to inquire whether the amount of the coloration is correlated with the age of the host. I suppose that we are justified in using the size of the shell as a criterion of the age of the animal, and if so, then we must conclude that the amount of the coloration is not in any way correlated with the age of the shell. It would be possible to find all sizes of shells with every stage of amount of color, — small ones in which there was little and much, and largest ones in which there was much. I have tabulated here a few measurements and amounts of coloration:

	Extreme Length of Valve.				
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
No red present	23	38	44	47	
Small umbonal patch	27	44			
Anterior patch no posterior	70	86			
Anterior and posterior patches . . .	28	61	64	72	80
Red general	49	50	67	73	

I am not able to state the percentage of Anodontas in which the red coloration is found; it is very prevalent, but not universal. Speaking very roughly, I should give it as my belief that at least 75 per cent of the animals at Chautauqua are affected, and I feel tempted to put the estimate even higher, for it is not at all frequently that one runs across a specimen in which the shell is absolutely normal.

It is an interesting and striking fact that the presence of the parasites is practically confined to the Anodontas. There are several species of Unio found in company with Anodonta, and these have all of them been carefully examined for the fluke. A single case of it has indeed been found,[1] — a specimen of *Unio edentula*, in which the shell exhibited the red coloration and flukes were found which seemed to be identical with those so commonly found in *Anodonta plana*. This case

[1] By Mr. R. H. Johnson.

is, however, the only one found after opening dozens, if not hundreds, of Unios.

II. Nature of the Coloration.

In order to determine, if possible, the exact relation of the color deposit to the shell, I made a vertical section of the shell, by sawing out a piece and then grinding it down to the necessary thinness on a stone. A view of this section is given in Fig. 2. The three usual layers of the shell are seen; *viz.*, the epidermis, on the outside; the prismatic layer beneath this, in which vertical prisms are distinctly visible; and the inner nacreous layer. The nacreous layer, however, is rather distinctly divided into two portions, a deeper, normal layer closely laminated and not colored, and an outer layer chiefly composed of the same material, but containing the red coloration in addition. The red is not a separate and distinct material mixed with an equally separate nacreous stuff, but the two are so intimately blended that it is impossible to show the red coloration in a black and white drawing. It thus appears to be rather a modification of the nacre, or at any rate a material dissolved in it, than any separate deposit.

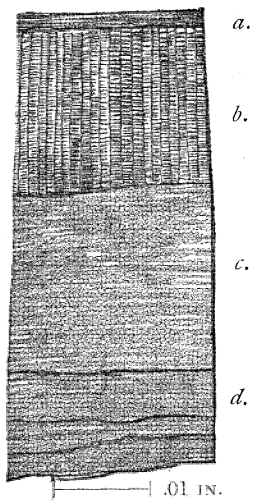

FIG. 2. — Cross-section of shell of Anodonta through a thick deposit of red material, showing its nacreous character. *a* = epidermis; *b* = prismatic layer; *c* = nacre; *d* = deposit due to the fluke.

Surface views of the red deposit were also studied. They were obtained by first decalcifying the shell in dilute hydrochloric acid, after which the lamination of the nacreous portion became very distinct and permitted me to tear off very thin pieces of considerable size. Such pieces under the microscope do not show any distinct colored material; they are structureless membranes which either are colorless or else they are salmon tinted. But in no case was I able to find any distinct and separate deposit. These facts are correlated with the

nacreous luster of the shell over the points of red coloration, and they indicate, not a separate layer of red deposited on the nacre, but the secretion of some soluble material which is poured out with the nacreous secretion and hardened with it.

III. RELATION OF THE PARASITES TO THE SHELL.

The parasites concerned in the red coloration are found attached by their suckers to that surface of the mantle which is toward the shell. Their home is thus in a perfectly closed chamber out of direct contact with the water, which is the home of the host and remote from sources of food such as are usual to parasites of their kind. Though I have carefully searched for them in other portions of the Anodontas, I have never been able to discover any in any other part of the host. I have not been able to find any evidence to show that the parasites enter the host with the in-going water and then bore through the tissues to find their place of residence. I have examined the surface of the mantle carefully to find any modification which its surface might have undergone, but without finding anything that could be assigned to the flukes. I have also sectionized the mantle for indications of flukes, but have not found any traces of them there. I am, therefore, inclined to believe that they reach their home by inserting themselves at the margin between the shell and the mantle.

The parasites are found in the red-colored shells almost without exception, and are always found directly at the points of coloration. In some cases there are a very few flukes, and these are umbonal in position or there is a line of them in the anterior line of coloration. Usually in shells with much color there are a great many flukes, and generally the flukes are found to agree in number and position with the red. I have found some highly colored shells in which there were no flukes, and concluded that they had migrated. I have also examined shells in which there was no coloration without finding any flukes. So that it can be said that the flukes are constantly present in colored shells, and that they are located at the points of coloration. I have found a very few shells

with much color and no flukes; these cases have not been numerous. I have interpreted them to mean that the flukes have migrated in search of another host.

The constant occurrence of the flukes in correlation with the coloration seems to be sufficient evidence to justify the conclusion that they are the cause of the coloration, but when one attempts to go beyond this point and determine the exact relation that exists, one reaches a realm of speculation. It seems to me probable that the red color is a modification of the secretion of the mantle due to the irritation by the flukes. I have not been able to find out why the flukes collect as they do, and am inclined to suppose some attraction, perhaps of food, which draws them to the points where they are habitually found.

IV. Facts about the Parasites.

The parasites present some points that are of interest to the student of the trematodes. Many specimens were examined alive under compression, and many others were studied in total preparation of specimens that had been fixed in corrosive sublimate solution and carried up through the alcohols and cedar oil to Canada balsam. One specimen was sectioned serially and the series carefully studied; the following description rests on these three modes of study.

A view showing the points observed on the living specimen and on the total preparation is shown in Figs. 3 and 4. The total length of the body in the living fluke was variable, according to its state of contraction, and it presented every condition between a broad and short form and a very long and slender one. The length of the preserved specimens is .04 of an inch. A living specimen under slight compression from the weight of the cover glass measured .054 inches.

All the structural features of the parasite are indicated in the accompanying text-figures, so that only a brief mention of them is necessary. The outline of the body is broadly elliptical and there is no distinction into regions. The outer skin is everywhere, except the suckers, entirely simple, and I did not see any conspicuous cuticle; there are no spines of any kind visible in any portion of the skin.

Two very distinct suckers are present; the anterior one surrounding the mouth is considerably smaller than the ventral, and located at the extreme anterior end of the body. The ventral sucker is very large and prominent. It is on the level

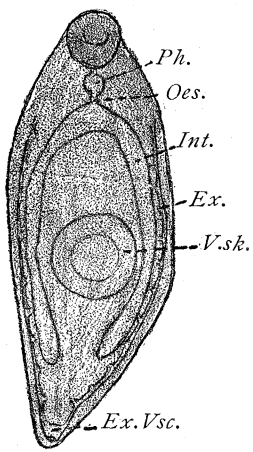

FIG. 3.

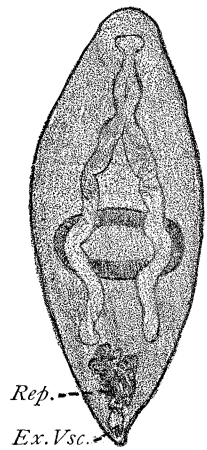

FIG. 4.

FIG. 3. — Drawing of the distomid, from life.

FIG. 4. — Drawing of the distomid, from a total preparation in balsam after corrosive sublimate and borax-carmine.

of the surrounding surface, is slightly wider than half the diameter of the body at its level, and is located very slightly behind the center of the ventral surface.

The body wall is as usual; its musculature can be seen in sections, greatly increased in amount at the suckers, and bounding the customary parenchymatous filling of the body cavity.

The alimentary organs are considerably differentiated. The pharynx closely follows the mouth; its wall is much thickened, and internally it is distinctly lined with nucleated columnar cells. The alimentary passage divides immediately behind the pharynx, without any intermediate oesophagus, into two intestinal tubes. These are entirely simple, and present an anterior portion lined with taller glandular cells shading posteriorly into a part, bounded by a flat-celled epithelium. So that in total mounts the anterior portion is dark and the hinder portion is clear and bladdery.

The excretory system consists of a terminal pore and a vesicle, into which in the living specimen two lateral passages are seen to lead. These lateral vessels (Fig. 3) in living specimens were traced forward where they were lost. They were ciliated so as to produce a current toward the terminal pore, and small side branches were seen which were lost in the parenchyma. Indications of a second similar and probably excretory vessel were seen at the hinder end of the body in compressed living specimens, but I was not able to see its connection (if it has one) with the more distinct lateral vessel already mentioned. In the preserved specimens (Fig. 4) the only portion of the excretory system that was visible is the terminal vesicle and the pore. The lateral vessels do not show; doubtless they are too delicate and similar to the parenchymatous cells to be distinguishable from them. I have not been able to recognize any nerves, and there are no noticeable sense organs on the surface of the body.

The reproductive system has not developed; there is, however, in the posterior end of the body in front of the vesicle of the excretory system a mass of undifferentiated cells. They are clearly seen in total preparations where they are deeply stained, and in contrast with the looser surrounding tissues. The cells of this mass are seen in sections as spherical objects with a large central nucleus, and the mass is clearly a mass of undifferentiated cells. Their future destination is not determined, but it seems very probable that it is the "Anlagen" of the reproductive organs. The gonads in some of the distomids are posterior in position, though the passages may run forward and open anteriorly.

V. Theoretical Points.

Since the reproductive organs are not as yet developed in these flukes, it is impossible to identify them by means of anatomical data; and since the case has not been reported heretofore, no light is thrown on the matter from the work of other observers. There seems, however, no doubt that the animal is of the family Distomidae, and it seems not unlikely

to belong to the genus Distomum. It seems to be a case of arrested development of the final form. The inactivity of the germinal tissue is indicated by the fact that no advance is noticed during the six weeks in which they have been observed, and by the fact that mitotic figures are not seen in the presumably germinal tissue noted above. The form of the body is clearly that of the final stage, and not a larval form. There is no evidence that I know of to show what the earlier stages are, but they are apparently passed in some other host; and the same is true of the final mature form, the Anodonta seemingly serving as a sort of halting place in which the flukes pass a certain period. It is also peculiar to find a distomid in this stage of its life history in an invertebrate host. The long list of hosts for distomids given in Bronn, '93, does not mention a single instance in which the mature form of the fluke is found in an invertebrate, though Aspidogaster is so found; but in that case there is only one host. It is also unique, as far as I have been able to learn, to find a distomid that is not located parasitically on some portion of the alimentary tube or on some hollow organ connected with it. Here, however, the fluke has no such relation. I have inferred from the restriction of the fluke to Anodonta, when there are so many allied animals at hand, that there is a close relation between the two, doubtless for the benefit of the fluke, and that when the case is better understood we shall find that the residence in Anodonta in this situation is an essential middle part of a life history, both ends of which are at present unknown or unrecognized in connection with this part.

I shall hope during the coming summer to make further observations upon these parasites and to plan for Anodontas being sent to me at regular intervals during a year, so that careful examinations can be made extending over a longer period. Also it is hoped to make examinations of the mussels at a number of different points on the lake. In the meantime it seems to be worth while to publish the results thus far obtained.

HAMLINE UNIVERSITY BIOLOGICAL LABORATORY,
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